

Oxytocin Dysregulation in Critically Ill Newborn Foals

Honors Research Thesis

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ABSTRACT

Sepsis is the leading cause of mortality among foals less than one week of age. Previous studies indicate elevated oxytocin in response to sepsis associated stress and decreased concentration with neurological dysfunction in critically ill human neonates, but limited information exists in newborn foals. Neonatal maladjustment syndrome (NMS) occurs in foals during or shortly after parturition, is often associated with septicemia, and is characterized by abnormal neurologic behavior, loss of suckle reflex, depression, and seizures. Objectives of this project were to measure oxytocin concentrations in neonatal foals and examine its association with severity of NMS and likelihood of survival. Newborn foals were categorized into 3 groups based on severity of illness: septic, sick non-septic (SNS), and healthy. Foals diagnosed with NMS were included. Plasma oxytocin concentrations were measured by enzyme immunoassay. Oxytocin concentrations were decreased in septic compared to healthy foals but increased in NMS compared to healthy foals ($P < 0.05$). In our study, decreased oxytocin concentrations were associated with septicemia and mortality, while increased oxytocin was linked to NMS. This is the first study to measure oxytocin in sick newborn foals and to demonstrate an association between this hormone with disease and outcome. This information provides additional insight on the pathogenesis of sepsis and neurological function in newborn foals.

INTRODUCTION

Sepsis is a condition where bacteria can multiply in the blood and set up widespread infection throughout the body of young foals. Septic foals have varying levels of survival depending on the severity and duration of infection. Initial infection is often followed by further complications, including septic shock, multiple organ dysfunction syndrome, and hormonal dysregulation. Previous studies have demonstrated an association of hypothalamus-pituitary-adrenal axis (HPAA) dysfunction and increased mortality rate in septic foals.

In response to stress, the hypothalamus releases CRH (corticotropin-releasing hormone), which stimulates the pituitary gland to secrete ACTH (adrenocorticotrophin). ACTH acts on the adrenal gland to release cortisol and aldosterone. In addition to CRH, the hypothalamus releases oxytocin that is stored in the pituitary gland. Oxytocin has a number of functions, many of which are associated with delivery and release of milk from mammary glands. This hormone also appears to be linked with trust and social behavior in people and other species (Toribio 2011). The role of oxytocin in response to sepsis associated stress is well documented in critically ill human neonates, but limited information exists in newborn foals.

Neonatal maladjustment syndrome (NMS), also known as dummy foal syndrome or hypoxic ischemic encephalopathy (HIE), occurs in foals during or shortly after parturition and is often associated with septicemia. The syndrome is characterized by abnormal neurologic behavior, loss of suckle reflex, depression, and seizures. The exact cause of NMS has not yet been determined; therefore association between oxytocin concentration and neurological abnormalities remains to be evaluated.

In 2013, Dembek et al. conducted research on the interaction between the renin-angiotensin-aldosterone system (RAAS) and hypothalamic-pituitary-adrenal axis (HPAA) in

septic, sick non-septic, and healthy foals. The authors hypothesized that activation of RAAS, as well as decreased levels of aldosterone concentration, hormone ratios, and electrolyte concentration, is associated with increased mortality rate in foals. The study of Dembek et al., (2013) collected clinical history reports and blood samples of a total of 167 foals. A sepsis score was calculated for each foal. The blood samples were analyzed to measure plasma renin activity and angiotensin-II, aldosterone, ACTH, and cortisol concentrations. Results showed increased concentrations of angiotensin-II, aldosterone, ACTH, and cortisol and decreased concentration of WBC, total protein, and IgG in septic foals compared to healthy foals. Positive correlation was found between aldosterone and potassium, creatinine, and BUN concentrations. The authors conclude that in sick foals, RAAS and HPA axis are activated, with RAAS activation characterized by increased concentrations of angiotensin-II and aldosterone. They also noted that more studies were needed on foal adrenal response to stimuli to deepen the understanding of septic endocrine activity (Dembek et al., 2013).

In 2008, Hurcombe et al. conducted research to determine the endocrine response of neonatal foals to septicemia and its influence on survival rates. They hypothesized that blood concentrations of each variable (arginine vasopressin, adrenocorticotropic hormone, and cortisol) would be higher in septic foals. The group collected samples of full-term sick, septic, and healthy foals. They used a solid phase, double antibody commercial radioimmunoassay to measure AVP concentrations, and they used a human-specific immunoradiometric assay to measure ACTH concentrations. Means, medians, and ranges were calculated for all values, and normality was evaluated using the Shapiro-Wilk statistic. The team also compared hormone concentrations between blood culture positive and blood culture negative septic foals. They found severity of sepsis to be highly associated with likelihood of survival. Increase in ACTH and AVP

concentrations were associated with mortality and no significant difference in cortisol levels. These findings were similar to previous study results for human hormone concentrations. However, in some studies of human adults, septicemia was associated with decreased concentrations of ACTH and cortisol. Their findings support the conclusion that insufficiency of the adrenal gland in septic foals is one of the main factors leading to mortality. Where hormone response was appropriate, systemic perfusion impairment decreased survival likelihood. The researchers concluded that multiple nonendocrinologic variables were associated with the survival of critically-ill foals, despite varying hormone levels and proper function of the hypothalamic-pituitary-adrenal axis (Hurcombe et al., 2008).

In 1998, Modahl et al. conducted a study on oxytocin concentrations in autistic children. The group used a radioimmunoassay to measure oxytocin in a group of 29 autistic children and 30 children without autism who were the same age. The results demonstrated significantly higher concentrations in the children without autism and elevated scores in those with more advanced social and developmental traits. The results also indicated that oxytocin concentrations increased with age in children without autism but failed to do so in those diagnosed with autism. The researchers state that the results show a possible association between oxytocin abnormalities and autism, but further knowledge of this hormone function should be obtained before coming to a final conclusion. Although this study was conducted on humans, possible association between oxytocin and autism may indicate a similar association between oxytocin and NMS in foals (Modahl et al., 1998).

Problem Identification and Justification

Septicemia is the leading cause of mortality among newborn foals. Diagnosis can be difficult, with a large range of observable symptoms. Oftentimes, foals die or are euthanized

before proper treatment can be administered. Superior knowledge of the endocrine response system and hormone associations would improve treatment options and survival rate in critically ill foals (Sepsis in Foals). Increased research on the endocrine system of neonatal foals would enhance the understanding of the role of hormone regulation in the pathogenesis of foal sepsis and NMS. More frequent monitoring of oxytocin could be used to indicate development of NMS, and action could quickly be taken to prevent onset of severe symptoms. If decreased oxytocin exhibits association with NMS, hormone concentration could be increased to stabilize the endocrine system of equine neonates. Treatment could be implemented to normalize hormone concentrations that fluctuate due to sepsis.

Furthermore, findings will potentially prevent millions of dollars in losses to the US equine industry through increased survival rate and decreased medical bills. Currently, information regarding the endocrine response to critical illness in foals is extremely limited. With a more comprehensive understanding of oxytocin concentration, evaluation of novel therapy methods will become much more competent.

Objectives and Hypothesis

The objective of this project was to investigate concentration of oxytocin in critically ill and healthy foals. We also examined associations of oxytocin with septicemia and likelihood of survival. Furthermore, we analyzed any correlation between NMS and oxytocin concentration.

We hypothesized that septic foals will have increased oxytocin concentration compared to healthy foals. We also proposed that foals with NMS will have decreased oxytocin compared to healthy foals.

PROCEDURES AND METHODOLOGY

We conducted a prospective, randomized study of three groups of foals: 37 septic, 14 sick non-septic (presented for other diseases such as lameness or meconium impaction) and 10 healthy foals of less than a week of age. Blood samples were collected on admission to the hospital (Rood and Riddle Equine Hospital, Hagyard Equine Medical Institute, and Ohio State University Galbreath Equine Center). Samples were processed immediately after collection, and serum and plasma were stored in -80C until analysis. Clinical information such as history, physical examination, blood work, diagnosis, treatment and outcome was obtained from medical records. The clinical information, physical examination, and historical data given were then used to assign each foal a sepsis score. Scores greater than or equal to 11 indicated a septic foal, and scores lower than 11 were considered to be non-septic. Foals were also assigned a survival score, ranging from 1 to 7, to predict likelihood of survival. We measured concentrations of oxytocin from plasma samples using a DetectX Oxytocin Enzyme Immunoassay Kit (Arbor Assays).

Statistical Analysis

Statistical analysis was performed to interpret results, with the group of healthy foals used as the control. A Shapiro-Wilk test was performed to assess data normality. The data sets were found to be not normally distributed. Mann-Whitney U test was used for comparison of oxytocin concentrations between foals with and without NMS. Kruskal-Wallis ANOVA was used to compare variables between the healthy, SNS, and septic groups. When comparing sepsis scores between septic and SNS foals, results with a P-value less than 0.01 were considered statistically significant. When comparing oxytocin concentrations and survival score between groups, results with a P-value less than 0.05 were considered statistically significant. Data

analysis was carried out using Microsoft Excel and GraphPad Prism. Data is presented in the graphs as median and interquartile range.

RESULTS

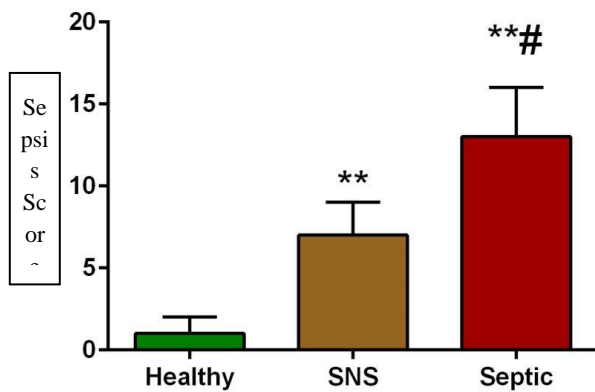


Figure 1. Sepsis scores of healthy, sick non-septic, and septic foals. ** = statistically different compared to healthy at $P < 0.01$; # = statistically different compared to SNS at $P < 0.01$

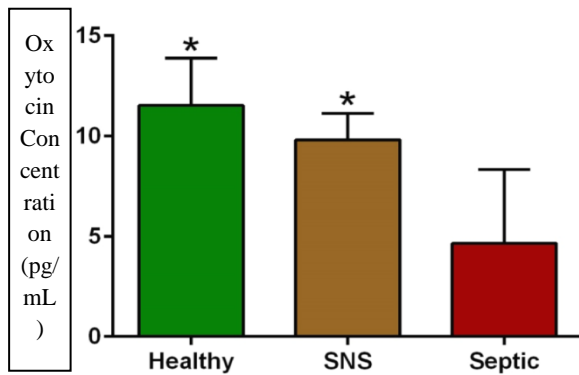


Figure 2. Oxytocin concentration (pg/mL) of healthy, sick non-septic, and septic foal groups. * = statistically different compared to septic at $P < 0.05$

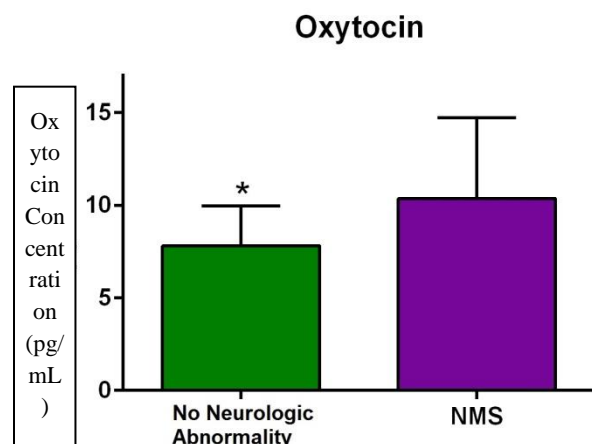


Figure 3. Oxytocin concentrations of foals with no neurologic abnormality in the healthy, sick non-septic, and septic groups compared to foals diagnosed with NMS. * = statistically different at $P < 0.05$

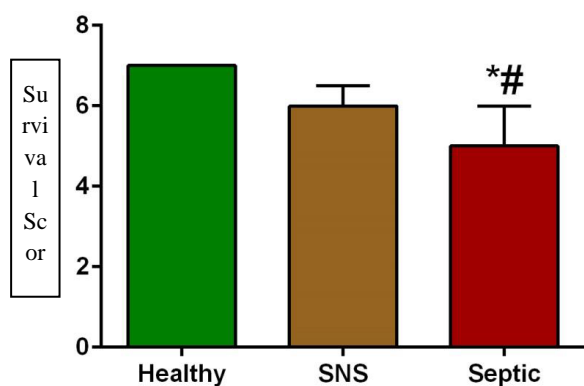


Figure 4. Survival score of healthy, sick non-septic, and septic foals. * = statistically different compared to healthy at $P < 0.05$, # = statistically different compared to septic at $P < 0.05$

Septic foals had greater sepsis scores than SNS foals, and both septic and SNS foals had greater sepsis scores compared to healthy foals (Figure 1; $P < 0.01$). The median score for foals with septicemia was 13, compared to 7 for sick foals without septicemia, and 1 for healthy foals. Plasma oxytocin concentrations were increased in healthy and SNS foals compared to septic foals. The median oxytocin concentration for septic foals was 6.5 pg/mL, whereas healthy foals had a median value of 11.5 pg/mL (Figure 2). Median plasma oxytocin concentration in hospitalized foals diagnosed with NMS was 13.4 pg/mL compared to 6.8 pg/mL in foals without

NMS (Figure 3). Survival score was decreased in septic foals, with a median score of 4, compared to healthy and SNS foals, with median values of 7 and 6, respectively. There was no difference between healthy and SNS foals (Figure 4).

Discussion

Limited information exists describing oxytocin concentration in horses and other mammalian species. Average serum oxytocin concentration in monkeys is reported to be 33.6 ± 4.6 pg/mL (Arbor Assays, 2015). In humans, oxytocin concentration typically ranges from 10.8 to 70 pg/mL. Although proposed mechanisms of NMS include hypoxic and ischaemic events surrounding parturition, increased concentration of neurosteroids have been found in these foals which may indicate an alternative cause. If these neurosteroids cross the blood-brain barrier, they can have neuromodulatory effects including seizure activity and obtundation, common symptoms of NMS (Madigan et al., 2012). According to Madigan, behavioral abnormalities of foals with NMS resemble some behavior of children with autism. Oxytocin concentrations in children with autism have been found to be lower than those without autism (Modahl, 1998). In humans, increased oxytocin has been associated with enhanced feelings of trust, cooperation, and social interaction (Andari et al., 2010). The results from this experiment show increased oxytocin in NMS foals, which indicates that there is still a great amount of research that needs to be done surrounding the mechanism of oxytocin and the pathogenesis of NMS.

CONCLUSION

This preliminary work indicates that there may be an association between septicemia, NMS, and oxytocin concentrations in neonatal foals. The data suggests that oxytocin abnormalities may exist in foals with septicemia and foals with NMS. In septic foals, profound depression and abnormal behavior may result from decreased concentrations of oxytocin, in

addition to other factors. Further studies are needed to evaluate oxytocin therapies in critically ill foals.

This is the first study showing increased oxytocin concentrations in foals with NMS. In these foals, there may be some dysfunction in release or reception of oxytocin causing increased concentrations. Studies in other species indicate conflicting association between oxytocin and social and neurological abnormality. A larger number of foals will be analyzed to validate our findings in the upcoming year. This information provides additional insight on the pathogenesis of sepsis and neurological function in newborn foals. If results are repeated and strengthened, this could lead to discoveries on the pathogenesis of NMS, as well as more efficient diagnosis, treatment, and prevention of both diseases.

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